Reference: *Biol. Bull.* **169:** 246–255. (August, 1985)

5-HYDROXYTRYPTAMINE MEDIATES RELEASE OF MOLT-INHIBITING HORMONE ACTIVITY FROM ISOLATED CRAB EYESTALK GANGLIA

MARK P. MATTSON AND EUGENE SPAZIANI

Department of Biology, University of Iowa, Iowa City, Iowa 52242

Abstract

Neurosecretory cells in crustacean eyestalk ganglia produce a putative molt-inhibiting hormone (MIH) which directly suppresses production of the steroid molting hormone, ecdysone, by the peripheral Y-organs. Neurotransmitter mediation of MIH release from isolated eyestalk ganglia of the crab, *Cancer antennarius,* was explored using an MIH bioassay based upon *in vitro* inhibition of Y-organ ecdysteroid production by eyestalk ganglion-conditioned saline. The conditioned saline (0.01–1.0 eyestalk equivalent) inhibited Y-organ ecdysteroid production dose-dependently and reversibly, and the effect of the saline was specific as to conditioning tissue. Isolated ganglia released a significant portion of their MIH activity in 2-h incubations, but also retained a significant portion. 5-hydroxytryptamine (5-HT) enhanced MIH release at concentrations of 10^{-10} *M* to 10^{-6} *M*. Acetylcholine, dopamine, octopamine, norepinephrine, or gamma-aminobutyric acid (10^{-7} *M*–10^{-6} *M*) did not alter basal MIH release. The 5-HT precursor, 5-hydroxytryptophan (10^{-7} *M*), stimulated MIH release, while the tryptophan hydroxylase inhibitor, p-chlorophenylalanine (10^{-6} *M*), and the 5-HT receptor antagonist, cyproheptadine (10^{-7} *M*), inhibited MIH release to below basal levels. Apparently, 5-HT neurons provide excitatory input to MIH-containing neurosecretory cells.

Introduction

In decapod crustaceans, the steroidogenic Y-organs are the source of the molting hormone, ecdysone (Chang *et al.,* 1976; Chang and O'Connor, 1977). Eyestalk extirpation and implantation studies (Carlisle, 1957; Gersch *et al.,* 1977) and *in vitro* suppression of steroidogenesis in Y-organ tissue segments and dispersed cells by eyestalk extracts (Mattson and Spaziani, 1985a, b; Watson and Spaziani, 1985) indicate that an eyestalk factor (MIH) directly inhibits Y-organs. MIH originates in eyestalk neurosecretory cells (X-organs) with axonal endings that form a neurohemal organ, the sinus gland (SG) (see reviews by Kleinholz, 1976; Cooke and Sullivan, 1982).

Evidence exists that suggests a synaptic basis for control of neurohormone release from the X-organ-SG complex. Isolated X-organ-SGs change electrical activity in response to 5-hydroxytryptamine (5HT) and gamma-aminobutyric acid (GABA) (Nagano and Cooke, 1981). In addition, studies by Fingerman and co-workers on the release of chromatophorotropic hormones from eyestalks *in vivo* and *in vitro* in *Uca pugilator* suggest that 5HT, dopamine (DA), and norepinephrine (NE) induce release of a red pigment-dispersing hormone, red and black pigment-concentrating hormones,

Received 26 March 1985; accepted 15 May 1985.

Abbreviations: ACH, acetylcholine; CPH, cyproheptadine; DA, dopamine; GCS, eyestalk ganglion-conditioned saline; ESE, eyestalk equivalent; GABA, gamma-aminoisobutyric acid; 5-HT, 5-hydroxytryptamine; 5HTP, 5-hydroxytryptophan; MIH, molt-inhibiting hormone; NE, norepinephrine; OA, octopamine; PCPA, p-chlorophenylalanine; RIA, radioimmunoassay; SG, sinus gland.

and black pigment-dispersing hormones, respectively (Fingerman and Fingerman, 1977a; Fingerman *et al.*, 1981; Quackenbush and Fingerman, 1984). Higher-order neurons with specific neurotransmitter phenotypes apparently control release of specific peptides from neurosecretory cells in mammalian pituitary (Mathison, 1981; McCann, 1981) and insect brain and corpus cardiacum (Samaranoyaka, 1976; Orchard *et al.*, 1983).

We reported recently a formal bioassay for MIH based on suppression by eyestalk extracts of ecdysteroid production in isolated Y-organ quarters of the crab *Cancer antennarius* (Mattson and Spaziani, 1985a). Soumoff and O'Connor (1982) showed that isolated whole Y-organs are inhibited in a dose-dependent manner by SG-conditioned saline from *Pachygrapsus crassipes*. The present study combines the MIH bioassay and the conditioned medium technique to measure release of MIH activity from isolated eyestalk whole ganglia exposed to neurotransmitters and agents that alter transmitter synthesis or receptor action. The experiments provide evidence that transmitter-specific higher-order neurons intrinsic to eyestalk ganglia supply excitatory input to MIH-containing neurosecretory cells.

Materials and Methods

Female rock crabs, *Cancer antennarius* Stimpson, were obtained from Pacific Biomarine (Venice, CA) and Marinus, Inc. (Westchester, CA), and were maintained individually in compartments of water tables with constantly circulating, charcoal-filtered reconstituted sea water at 16–17°C. Crabs were fed fish three times weekly and were allowed to acclimate to their environment for at least two weeks prior to experimentation (Mattson and Spaziani, 1985c).

Eyestalks were cleaned with alcohol swabs, removed by cutting connective tissue and the optic nerve at the base, and transferred to sterile Pantin's saline (Pantin, 1934). The protruding stump of the optic nerve was grasped with a blunt forceps and the entire complex of optic ganglia (including the X-organ-sinus gland complex) was removed from the exoskeleton, freed of adhering connective and ommatidial tissue, and placed in saline (1 ganglia/100 μl saline) without (control) or with given concentrations of neurotransmitters or drugs. Contralateral ganglia from individual animals were randomly assigned to control and treatment plates to control for possible effects of interanimal variabilities in MIH neurosecretory activity. Incubations were at 20°C in an atmosphere of 50% oxygen/50% air with rotary shaking at 60 rpm for 2 h unless otherwise stated. In one experiment individual sinus glands were isolated by dissection and incubated under the above conditions. At the end of incubations the conditioned saline was removed, immersed in a boiling water bath for 2 min, and centrifuged at $1000 \times g$ for 10 min. The supernatant was removed, adjusted to the initial incubation volume with water, and stored frozen. 5HT, DA, NE, acetylcholine (ACH), octopamine (OA), GABA, 5-hydroxytryptophan (5HTP), p-chlorophenylalanine (PCPA), cyproheptadine (CPH), and reserpine were obtained from Sigma Chemical Co. (St. Louis, MO).

For the assay of MIH activity, activated Y-organs from 48 h de-eyestalked crabs were removed, quartered, and incubated for 24 h under conditions supporting maximal and sustained ecdysteroid production as previously described (Mattson and Spaziani, 1985a). Under these conditions Y-organ quarters produced between 90 and 130 pg of ecdysteroids/μg protein/24 h in all experiments in the present study. To Y-organ quarters incubated in 500 μl of medium (Medium 199 adjusted to crab serum osmolarity and supplemented 10% with fetal calf serum, and buffered with 2.0 g/l HEPES) was added 100 μl of saline (control) or of eyestalk ganglia-conditioned saline (GCS),

SG-conditioned saline, or saline containing neurotransmitters or drugs (controls for possible direct action of these agents on Y-organ ecdysteroid production). When consecutive incubations (with intervening medium changes) were done, Y-organ tissue was washed twice (10 min/wash) with 500 μl of medium between incubations.

At the end of incubations, medium was removed and stored at 4°C for ecdysteroid RIA. At the end of final incubations Y-organ tissue was processed for protein quantification. Procedures for ecdysteroid RIA and preparation of tissue for protein assay have been previously described (Mattson and Spaziani, 1985a). Protein was quantified by the Bradford (1976) method using BSA as standard. Ecdysone antiserum (antibody H21B; Horn *et al.*, 1976; a gift from W. E. Bollenbacher, Dept. of Zoology, Univ. of North Carolina, Chapel Hill), tritium-labeled ecdysone (specific activity 60 mCi/mmol; New England Nuclear, Bedford, MA), and ecdysone standards (Research Plus, Bayonne, NJ) were used for the ecdysteroid RIA. As Y-organs produce more than one ecdysteroid with affinity for the antiserum used (Watson and Spaziani, 1985a) values represent ecdysone equivalents. Intra- and interassay variabilities were 40 and 80 pg, respectively at an ecdysone dose of 1 ng. Conditioned saline, neurotransmitters, and drugs used did not interfere with ecdysteroid RIA.

MIH activity in GCS from neurotransmitter- or drug-treated ganglia was calculated as the ratio of 24 h ecdysteroid production of Y-organ quarters exposed to control GCS divided by those exposed to GCS from treated ganglia, $\times 100$. Control GCS from 2-h incubations inhibited Y-organ ecdysteroid production to 40–60% of Y-organ control levels in all experiments. Statistical comparisons were done using Student's *t*-test.

Results

Time course of release of MIH activity from isolated eyestalk ganglia and sinus glands

We previously showed that ecdysteroid production by Y-organ quarters is inhibited dose-dependently by MIH activity in eyestalk extracts (Mattson and Spaziani, 1985a). Similar results were obtained in a dose-response study with GCS (Fig. 1). MIH activity in GCS inhibited 24-h ecdysteroid production by Y-organ quarters dose-dependently at concentrations of 0.01 to 1.0 eyestalk equivalents (ESE). Inhibition to less than 60% of control levels occurred at a GCS dose of 1 ESE; half maximal inhibition was obtained with 0.01–0.1 ESE (Fig. 1). As Soumoff and O'Connor (1982) found that isolated sinus glands from *Pachygrapsus crassipes* released a maximal amount of MIH activity within 1 h, we compared levels of MIH activity (as measured by degree of inhibition of Y-organ ecdysteroid production) released over time from isolated sinus glands and entire eyestalk ganglia complexes. This experiment was designed to determine an incubation time period which would allow release of MIH, but also the retention of measurable amounts of MIH activity in ganglia. Figure 2 shows that 1 eyestalk equivalent (ESE) of GCS from 2-h and 8-h incubations significantly inhibited Y-organ ecdysteroid production to 53% and 31% of control levels, respectively. MIH activity in 2-h GCS was intermediate to, and significantly different from, activity in 30-min and 8-h GCS. One ESE of SG-conditioned saline from 30-min, 2-h, and 8-h incubations significantly suppressed Y-organ activity to near 30% of control levels. MIH activity released from SGs at all three incubation times was significantly greater than activity released from ganglia incubated for 30 min and 2 h, but was similar to MIH activity in 8-h GCS. As intermediate amounts of MIH activity were released from eyestalk ganglia incubated for 2 h, this incubation time was chosen for all subsequent experiments.

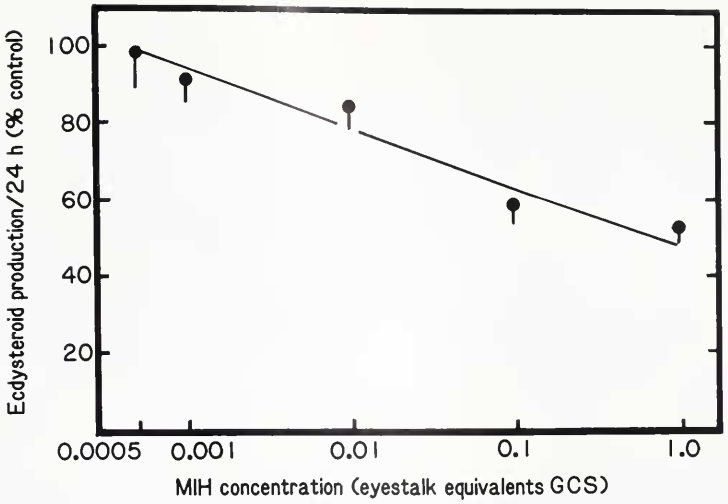

FIGURE 1. Dose-dependent inhibition of Y-organ activity by eyestalk ganglion-conditioned saline (GCS). Y-organ quarters were incubated 24 h with the stated concentrations (expressed as eyestalk equivalents ESE) of GCS and ecdysteroid production quantified by RIA. Points are each the mean and st. error of mean of 4 (0.0005 ESE) or 8 (0.001–1.0 ESE) incubations. Control Y-organs produced 128 ± 10 pg/μg gland protein of ecdysteroids. Inhibition was significant at GCS doses of 0.01 ESE or greater ($P < 0.05$–0.001).

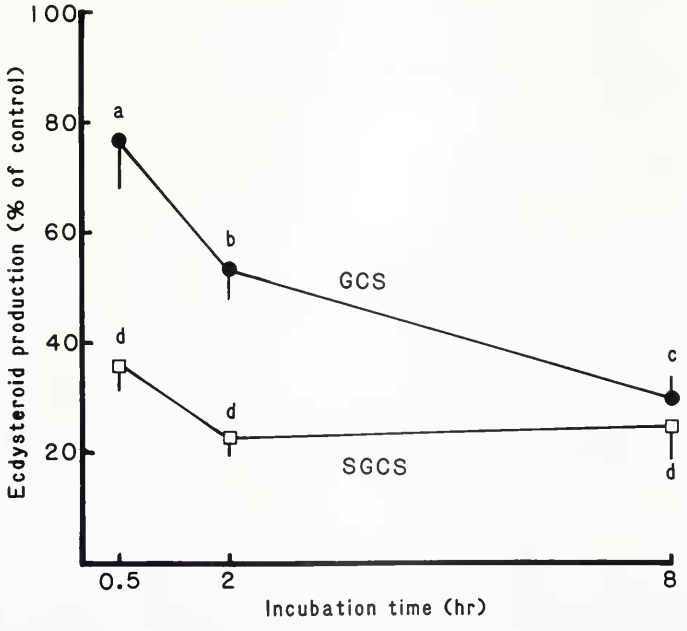

FIGURE 2. Effect of incubation time on release of MIH activity from isolated eyestalk ganglia and sinus glands. The tissues were incubated for the stated times in saline (1 ESE in 100 μl of saline), and MIH activity of the ganglion-conditioned (GCS)- and sinus gland-conditioned (SGCS)-salines determined by ability to inhibit ecdysteroid production by Y-organ quarters *in vitro* (24 h). Points are each the mean and st. error of mean of four incubations. Control Y-organ incubations produced 123 ± 18 pg of ecdysteroids/μg protein. a, $P < 0.001$ *vs* d; b, $P < 0.05$ *vs* a and c, $P < 0.01$ *vs* d; b, c and d, $P < 0.01$–0.001 *vs* control.

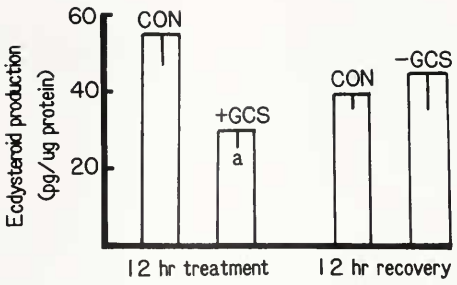

FIGURE 3. Reversibility of GCS-induced suppression of Y-organ function. Y-organ quarters incubated with (+GCS, 1 ESE) or without (CON) GCS for 12 h were washed twice (10 min/wash) with fresh medium and incubated an additional 12 h in the absence of GCS. Height of each bar and enclosed line is the mean and st. error of mean of four incubations. a, $P < 0.05$ *vs* CON.

Characterization of effects of GCS on Y-organ tissue ecdysteroid production

To determine whether GCS-induced inhibition of Y-organs was reversible, Y-organs were incubated for 12 h with 1 ESE of GCS, washed, and incubated an additional 12 h in the absence of GCS. Figure 3 indicates that Y-organ ecdysteroid production was significantly inhibited by GCS in the first 12-h incubation but was not significantly different from control ecdysteroid levels in the second 12-h incubation after GCS removal. To show that inhibition of Y-organ ecdysteroid production was specific for saline conditioned with eyestalk ganglia, saline conditioned with crab muscle or brain were tested for ability to alter Y-organ activity. Brain- and muscle-conditioned saline at concentrations equivalent to 1 ESE of GCS (equivalent wet weights/saline volume) did not significantly alter Y-organ ecdysteroid production in 24-h incubations, while GCS significantly suppressed ecdysteroid production to near 50% of control levels (Fig. 4).

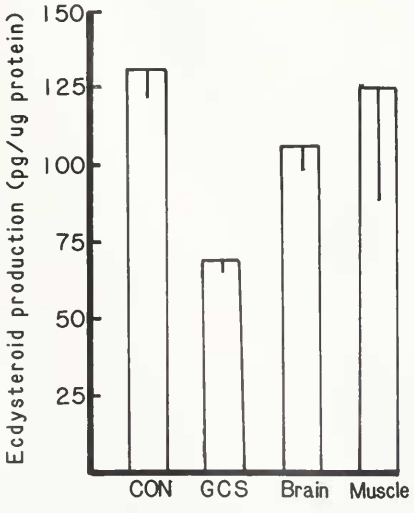

FIGURE 4. Tissue specificity of MIH activity released into saline. Ecdysteroid production of Y-organ quarters incubated for 24 h with 1 ESE of GCS or saline conditioned with crab brain or muscle (tissue weight/saline volume equal to 1 ESE) were compared. Bars and enclosed lines are the mean and st. error of mean of four incubations. GCS, $P < 0.01$ *vs* CON.

Effects of neurotransmitters on release of MIH activity from isolated eyestalk ganglia

Eyestalk ganglia were incubated for 2 h in saline containing a neurotransmitter, and MIH activity released into this saline was compared to that released into control saline. One ESE of GCS was used for control (basal) MIH release since it is clear from Figure 2 that the ganglion retained sufficient MIH activity in 2-h incubations to allow detection of changes in release of MIH activity caused by neurotransmitters. Control GCS inhibited Y-organs 40–60% in all experiments. Ganglia exposed to 10^{-7} *M* 5HT released significantly more MIH activity than control ganglia (Fig. 5). DA (10^{-6} *M*), NE (10^{-6} *M*), GABA (10^{-7} *M*), ACH, (10^{-7} *M*), and OA (10^{-6} *M*) had no significant effect on bioassayable MIH activity release from eyestalk ganglia. In control experiments in which neurotransmitters alone were added directly to cultured Y-organ tissue at concentrations equivalent to those in GCS it was found that DA (10^{-6} *M*) significantly inhibited ecdysteroid production to 40% of control levels (other neurotransmitters had no effect on Y-organ activity). However, when 10^{-6} *M* DA was incubated in saline alone for 2 h and the saline was carried through the usual boiling, centrifuging, freeze-thawing procedure for GCS (see Materials and Methods), this saline was ineffective in suppressing Y-organ ecdysteroid production. It was concluded, therefore, that DA does not affect MIH release.

Figure 6 shows that 5HT enhanced dose-dependently the release of MIH activity from isolated eyestalk ganglia. 5HT at 10^{-7} *M* caused maximal release of MIH activity; half maximal effect occurred with 5HT at approximately 10^{-10} *M*.

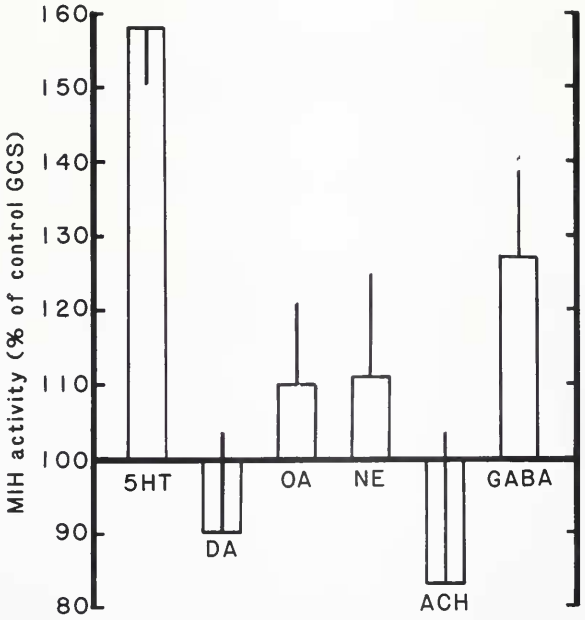

FIGURE 5. Effect of neurotransmitters on release of MIH activity from isolated eyestalk ganglia. MIH activity in 1 ESE of GCS from control (CON) or transmitter-treated ganglia in 2-h incubations were compared using the Y-organ bioassay for MIH. Control Y-organ tissue produced a mean of 95 pg of ecdysteroids/μg protein/24 h. Control GCS inhibited Y-organs 40–60% in all experiments. Bars and enclosed lines are the mean and st. error of mean of 8 (5HT) or 4 (other neurotransmitters) assays. Concentrations of neurotransmitters were 10^{-7} *M* for 5HT, gamma-aminobutyric acid (GABA), and acetylcholine (ACH); 10^{-6} *M* for dopamine (DA), norepinephrine (NE), and octopamine (OA). 5HT, $P < 0.05$ *vs* control (100%) GCS value.

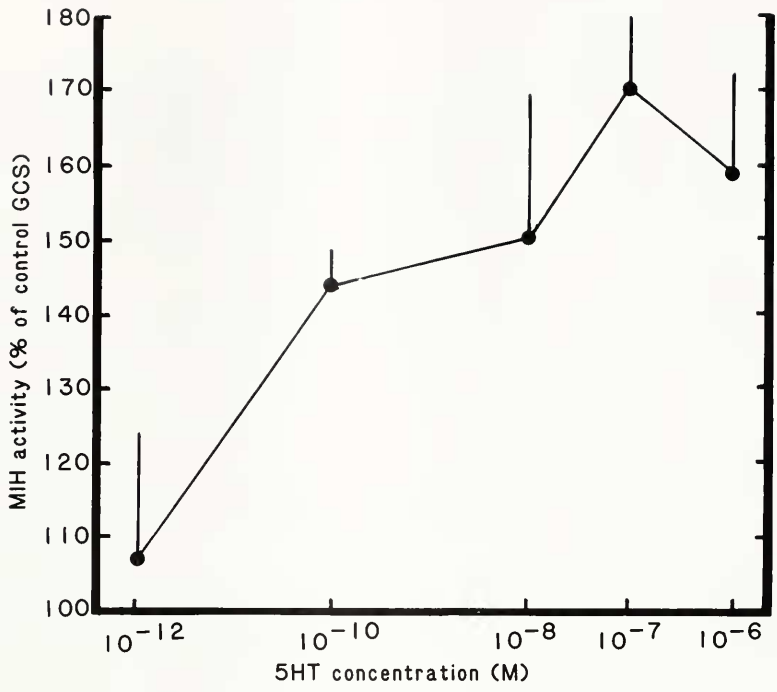

FIGURE 6. Dose dependency of 5HT-enhanced release of MIH activity from isolated eyestalk ganglia. MIH activities in 1 ESE of GCS from ganglia treated for 2 h with the given 5HT concentrations were measured using the Y-organ bioassay. Points are the mean and st. error of mean of 8 (10^{-7} *M*) or 4 (other 5HT doses) incubations. 5HT at doses of 10^{-10} *M* or greater significantly inhibited Y-organ ecdysteroid production ($P < 0.05$–0.02). Control Y-organ tissue produced 102 ± 20 pg of ecdysteroids/μg protein/24 h.

Effects of agents altering 5HT synthesis, release, or receptor activity on release of MIH activity

Isolated eyestalk ganglia exposed to the 5HT precursor 5HTP (10^{-7} *M*) released significantly more MIH activity (170%) than control ganglia (Fig. 7). GCS from ganglia treated with the tryptpophan hydroxylase inhibitor, PCPA (10^{-6} *M*) or the 5HT receptor antagonist, CPH (10^{-7} *M*), contained significantly less MIH activity (80%) than control GCS. Ganglia exposed to both 5HT and CPH (10^{-7} *M* each) showed no significant change in MIH release compared to control ganglia. The monoamine depletor, reserpine, at a concentration of 10^{-6} *M* did not have a significant effect on assayable MIH release.

DISCUSSION

A method for bioassay of MIH activity in crab eyestalk-conditioned saline (Figs. 1–4) has allowed assessment of neurotransmitter involvement in release of MIH activity and identification of 5HT as a stimulator of MIH release from the X-organ-SG neurosecretory complex in *Cancer antennarius* (Figs. 5–7). Release of maximal MIH activity from isolated SGs alone occurred within 30 min of incubation (Fig. 2), consistent with Soumoff and O'Connor's (1982) report that all detectable release of MIH activity from SGs of *Pachygrapsus crassipes* occurred in less than 1 h *in vitro*. In contrast, isolated eyestalk ganglia which contained intact X-organ-SG neurosecretory cells released MIH but also retained measurable MIH activity for several hours after

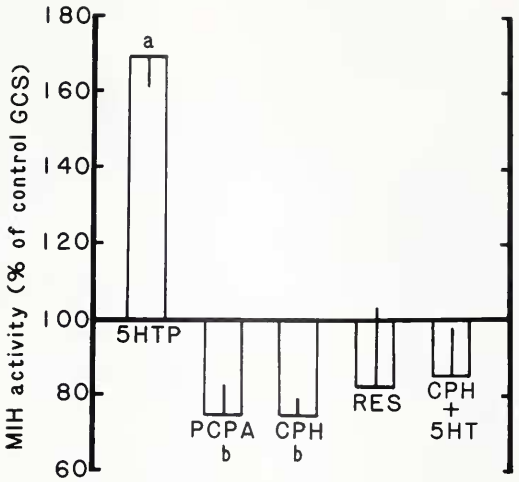

FIGURE 7. Effects of agents that alter 5HT synthesis, release, or receptor action on MIH activity released from isolated eyestalk ganglia. The Y-organ bioassay was used to compare MIH activities in 1 ESE of GCS from control (CON) or treated ganglia after 2 h incubations. Control Y-organ tissue produced a mean of 90 ± 13 pg of ecdysteroids/μg protein/24 h. Control GCS inhibited Y-organs 40–60% in all experiments. 5-hydroxytryptophan (5HTP) and cyproheptadine (CPH) concentration was 10^{-7} *M*; p-chlorophenylalanine (PCPA) and reserpine (RES), 10^{-6} *M*. For the experiment in which ganglia were exposed to both CPH and 5HT, ganglia were incubated in CPH-containing saline for 5 min prior to addition of 5HT. Bars and enclosed lines are the mean and st. error of mean of four assays. a, $P < 0.02$ *vs* control (100%) GCS value; b, $P < 0.05$ *vs* control.

removal to saline (Fig. 2). It is likely that severing X-organ axons during SG isolation induces MIH release. Doses of GCS and SG-conditioned saline capable of significantly inhibiting Y-organ activity (0.01 ESE, Fig. 1 and Soumoff and O'Connor, 1982) are an order of magnitude lower than doses of homogenized eyestalk extract required for inhibition (Mattson and Spaziani, 1985a; Watson and Spaziani, 1985); MIH activity appears to be lost in the extraction procedure, probably because of insufficient breakup of secretory granules. These results suggest that quantitative recovery of MIH may be obtained by collecting 8-h GCS, which contains MIH activity equal to that in SG-conditioned saline but does not require tedious dissection of SGs. The inhibitory effects of GCS on Y-organs were reversible and specific (Figs. 3, 4) consistent with previously reported effects of eyestalk extracts using the same bioassay culture system (Mattson and Spaziani, 1985a).

Within limits of sensitivity of our MIH bioassay, 5HT induces release of MIH activity from isolated eyestalk ganglia at concentrations of 10^{-10} *M* or greater, consistent with concentrations of neurotransmitters causing release of chromatophorotropic hormones from isolated eyestalks in *Uca pugilator* (Quackenbush and Fingerman, 1984). Our findings that the 5HT precursor, 5HTP, stimulates, while PCPA (an inhibitor of 5HT synthesis) or CPH (a 5HT receptor blocker) inhibits release of MIH activity (Fig. 7), suggest that neurons exist in eyestalk ganglia that provide 5HT input to MIH-containing cells. The effects of PCPA and CPH in lowering MIH release to below basal (control) levels suggest interference with endogenous 5HT. In addition, CPH blocked the stimulatory effect of exogenously applied 5HT, indicating a specific effect on 5HT receptors. Although a significant inhibition of release of MIH activity was not obtained with the monoamine depletor, reserpine, due to variability between ganglia, the mean level of MIH activity released by reserpine-treated ganglia was identical to levels released by PCPA-treated ganglia. Morphological studies indicate few or no

synapses on X-organ somata or axonal terminals in SGs, with many synapses localized to neurites (Cooke and Sullivan, 1982).

It is likely, therefore, that 5HT receptors antagonized by CPH (Fig. 7) are localized to neurites. Other neurotransmitters tested in the present study were ineffectual in altering release of MIH activity. This suggests that if higher-order neurons control the 5HT neurons providing input to MIH-containing cells they are either located elsewhere in the CNS or they are also serotonergic; intrinsic 5HT neurons may also be directly responsive to visual inputs (*e.g.*, light, movement).

In the course of control experiments we found that DA applied directly to cultured Y-organ tissue caused significant inhibition of ecdysteroid production; the processing procedure for GCS (see Materials and Methods) abolished this DA activity. DA was likely oxidized by the incubation, boiling process. We are currently exploring the direct action of DA on Y-organ tissue and preliminary evidence suggests that DA (as is the case for MIH activity, Mattson and Spaziani, 1985b) acts through the cyclic AMP second messenger system (unpub. obs.).

Evidence suggests that release of specific neurotransmitters by neurons impinging on neurosecretory cells results in release of specific neurohormones. NE and DA stimulate release of leuteinizing hormone-releasing hormone (Negro-Vilar *et al.*, 1979), while cholinergic and beta-adrenergic stimulation induce vasopressin release from hypothalamic-hypophyseal cells in mammals. Neurosecretion is induced by 5HT in insects (Scharrer and Wurzelmann, 1978), and hyperglycemic hormone and erythrophore-concentrating hormone are released from crustacean X-organ-SG cells in response to 5HT and DA stimulation, respectively (Keller and Beyer, 1968; Fingerman and Fingerman, 1977a). However, it is apparent that selective activation of subgroups of neurons with a particular transmitter phenotype must occur in order for specific neurohormones to be released in response to specific environmental stimuli. In crustaceans, 5HT induces release from eyestalks of red pigment-dispersing hormone (Fingerman and Fingerman, 1977a), hyperglycemic hormone (Keller and Beyer, 1968), and MIH (present study). Combined data from several laboratories suggests that independent groups of 5HT neurons may respond to different (or similar) environmental stimuli. Thus red pigment-dispersing hormone release is maximal in daylight (Fingerman and Fingerman, 1977b), while hyperglycemic hormone release is maximal in darkness (Dean and Vernberg, 1965). In addition, both hyperglycemic hormone (Keller, 1983) and MIH (Mattson and Spaziani, 1985c) are apparently released in response to environmental stressors. Control of release from neurosecretory cells by neurons containing classical neurotransmitters thus appears complex and future immunohistochemical, biochemical, and electrophysiological experiments will be required to identify and quantify neurosecretory cells in crustaceans containing specific hormonal products, to assess possible processing of larger precursor molecules, and to elucidate neural inputs controlling release. In the case of MIH definitive studies in these areas require isolation and purification of MIH and anti-MIH antibody production.

Acknowledgments

Portions of this work were presented at the 1984 annual meeting of the American Society of Zoologists, Denver, CO. This work was funded in part by NIH institutional grants 2T32MH5172-07 (M.P.M.) and AM 25295-06 (E.S.), and grant DCB 8408957 from the National Science Foundation (E.S.)

LITERATURE CITED

Bradford, M. M. 1976. A rapid and sensitive method for quantification of microgram quantities of protein using the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–253.

Carlisle, D. B. 1957. On the hormonal inhibition of molting in decapod Crustacea II. The terminal anecdysis in crabs. *J. Mar. Biol. Assoc. U. K.* **36:** 291–307.

CHANG, E. S., B. A. SAGE, AND J. D. O'CONNOR. 1976. The qualitative and quantitative determination of ecdysones in tissues of the crab, *Pachygrapsus crassipes,* following molt induction. *Gen. Comp. Endocrinol.* **30:** 21–33.

CHANG, E. S., AND J. D. O'CONNOR. 1977. Secretion of alpha-ecdysone by crab Y-organs *in vitro. Proc. Natl. Acad. Sci. U. S. A.* **74:** 615–618.

COOKE, I. M., AND R. E. SULLIVAN. 1982. Hormones and neurosecretion. Pp. 205–290 in *The Biology of Crustacea,* Vol. 3, D. Bliss, ed. Academic Press, New York.

DEAN, J. M., AND F. J. VERNBERG. 1965. Variations in the blood glucose level of crustacea. *Comp. Biochem. Physiol.* **14:** 29–34.

FINGERMAN, M., AND S. W. FINGERMAN. 1977a. Antagonistic actions of dopamine and 5-hydroxytryptamine on color changes in the fiddler crab *Uca pugilator. Comp. Biochem. Physiol.* **58C:** 121–127.

FINGERMAN, S. W., AND M. FINGERMAN. 1977b. Circadian variations in the levels of red pigment-dispersing hormone and 5-hydroxytryptamine in the eyestalks of the fiddler crab, *Uca pugilator. Comp. Biochem. Physiol.* **56C:** 5–8.

FINGERMAN, M., M. M. HANUMANTE, S. W. FINGERMAN, AND D. C. REINSCHMIDT. 1981. Effects of norepinephrine and norepinephrine agonists and antagonists on the melanophores of the fiddler crab, *Uca pugilator. J. Crust. Biol.* **1:** 16–27.

GERSCH, M., K. RICHTER, AND H. EIBISCH. 1977. Studies on the characterization and action of the molt-inhibiting hormone (MIH) of the sinus gland in *Orconectes limosus. Zool. Jb. Physiol.* **81:** 133–152.

HORN, D. S., J. S. WILKE, B. S. SAGE, AND J. D. O'CONNOR. 1976. A high affinity antiserum specific for the ecdysone nucleus. *J. Insect Physiol.* **22:** 901–905.

KELLER, R., AND J. BEYER. 1968. Zur Hyperglykämichen Wirkung von Serotonin und Augenstielextrakt beim Flusskrebs *Orconectes limosus. Z. Vergl. Physiol.* **59:** 78–85.

KELLER, R. 1983. Biochemistry and specificity of the neurohemal hormones in Crustacea. P. 131 in *Neurohemal Organs of Arthropods,* A. P. Gupta, ed. Charles C. Thomas, Springfield, Illinois.

KLEINHOLZ, L. H. 1976. Crustacean neurosecretory hormones and physiological specificity. *Am. Zool.* **16:** 151–166.

MATHISON, R. 1981. Regulation of secretion from the neurohypophysis. Pp. 167–175 in *Neurosecretion,* D. S. Farner, and K. Lederis, eds., Plenum Press, New York.

MATTSON, M. P., AND E. SPAZIANI. 1985a. Characterization of molt-inhibiting hormone action on crustacean Y-organ segments and dispersed cells in culture, and a bioassay for MIH activity. *J. Exp. Zool.* (in press).

MATTSON, M. P., AND E. SPAZIANI. 1985b. Cyclic AMP mediates negative regulation of Y-organ ecdysteroid production. *Mol. Cell. Endocrinol.* (in press).

MATTSON, M. P., AND E. SPAZIANI. 1985c. Stress reduces hemolymph ecdysteroid levels in the crab: mediation by the eyestalks. *J. Exp. Zool.* **234:** 319–323.

MCCANN, S. M. 1981. Monoaminergic and peptidergic control of anterior pituitary secretion, Pp. 139–166 in *Neurosecretion.* D. S. Farner, and K. Lederis, eds. Plenum Press, New York.

NAGANO, M., AND I. M. COOKE. 1981. Electrical activity in the crab X-organ-sinus gland system: site of inititation, ionic bases and pharmacology. P. 501 in *Eighth International Symposium on Neurosecretion,* K. Lederis and D. Farner, eds. Plenum Press, New York.

NEGRO-VILAR, A., S. R. OJEDA, AND S. M. MCCANN. 1979. Catecholaminergic modulation of leuteinizing hormone-releasing hormone release by median eminence terminals *in vitro. Endocrinology* **104:** 1749–1755.

ORCHARD, I., R. P. REUGG, AND K. G. DAVEY. 1983. The role of central aminergic neurons in the action of 20-hydroxyecdysone on the neurosecretory cells of *Rhodnius prolixus. J. Insect Physiol.* **29:** 387–391.

PANTIN, C. F. A. 1934. On the excitation of crustacean muscle. *J. Exp. Biol.* **11:** 11–27.

QUACKENBUSH, L. S., AND M. FINGERMAN. 1984. Regulation of the release of chromatophorotropic neurohormones from the isolated eyestalk of the fiddler crab, *Uca pugilator. Biol. Bull.* **166:** 237–250.

SAMARANAYAKA, M. 1976. Possible involvement of monoamines in the release of adipokinetic hormone in the locust *Schistocerca gregaria. J. Exp. Biol.* **65:** 415–425.

SCHARRER, B., AND S. WURZELMANN. 1978. Neurosecretion. XVI. Experimentally induced release of neurosecretory material by exocytosis in the insect *Leucophaea maderae. Cell Tissue Res.* **190:** 173–180.

SOUMOFF, C., AND J. D. O'CONNOR. 1982. Repression of Y-organ secretory activity by molt-inhibiting hormone in the crab, *Pachygrapsus crassipes. Gen. Comp. Endocrinol.* **48:** 432–439.

WATSON, R. D., AND E. SPAZIANI. 1985. Biosynthesis of ecdysteroids by crab Y-organs, and eyestalk suppression of cholesterol uptake and secretory activity, *in vitro. Gen. Comp. Endocrinol.* **58** (in press).